One-pot approach synthesizing and characterization of Random copolymerization of Ethyl Acrylate-co-methyl methacrylate with broad range of glass transition temperature onto Collagen

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ABSTRACT

Collagen is a natural polymer that cannot be applied freely to specific end uses due to its inherent drawbacks. Grafting polymerization of Methyl Methacrylate-co-Ethyl Acrylate was applied to modify the surface of acid soluble collagen(ASC). The main objective of this work is not only reducing the hydrophilic behavior of collagen on which has been concentrated so far, but also successfully showed that the introduced co-monomers onto ASC can alter the thermal behavior of the resulted copolymer. The level of branched copolymer significantly influenced the initial viscosity in studied co-monomer feed ratios. The graft polymerization of collagen demonstrated the meaningful change in conductivity value of branched copolymer, where the copolymer in side chain with low dielectric constant covalently bonded onto the ASC. The increase in the co-monomer feed ratio had no significant effect on conductivity value of copolymer, afterwards. The novelty of this work was determined to achieve the new copolymer onto the backbone of collagen to be used in specific thermally stabled end uses that the sufficient chain entanglements improve the flexibility of final product.

KEYWORDS: Collagen, Grafting polymerization, Polymer architecture, Branched copolymer, Co-monomer, Ethyl acrylate, methyl methacrylate

Abbreviations:

(ASC) Acid Soluble Collagen

(ASC-g-P(MMA-co-EA)) Acid Soluble Collagen-g-Poly(Methyl Methacrylate-co-Ethyl Acrylate)

(P(MMA-co-EA)) Poly(Methyl Methacrylate-co-Ethyl Acrylate

(DSC) Differential scanning calorimetry

1- INTRODUCTION

An idealistic approach in modern life is gradual replacement of natural resources with synthetics for achieving a sustainable living environment[1,2]. In materials and particularly in polymers, it is essential to modify their specific properties to desired specifications designed for specific end uses. There are several methods for modifying polymers[1,2]. Grafting polymerization is a method in which the surface of the main polymer is modified through covalently bonded monomer(s) onto its chain[2,3]. This sort of modification is achieved with the simultaneous or sequential arrangement of the participated monomer(s)[2,4-6]. Two or more different compatible monomers are grafted into the main polymer chain to gain the desired property[1,7].

Collagen is a natural polymer that cannot be applied freely to specific end uses due to its inherent drawbacks such as poor mechanical properties and fast degradability[2,8,9]. Since collagen can be modified into physiologically tolerable compounds, its properties is being investigated to improve its performance in new applications[10]. This biopolymer has been attempted to be modified by graft polymerization to benefit from its natural properties whilst at the same time, adding value by the monomer(s) that is introduced to the main chain[2,11].

In the past, the effects of various parameters in grafting polymerization onto biopolymers have been investigated to form corresponding radical on the chain [12]. Those investigations consist of applying varied sort of vinyl monomers such as butyl acrylate[13] , ethyl acrylate[6] , methyl acrylate[14] , acrylonitrile[15] , meth acrylonitrile[16,17] and methyl methacrylate [18], applying different dosage of initiators [19]. Also, the graft performance has been attempted to be optimized, based on the reaction parameters such as time, temperature and monomer feed ratio. To date the main objective of most investigations in this field have been focused on reducing the hydrophilic behavior and possessing the collagen property that mimics the native soft tissue with main uses in drug delivery[4,20], filtration[21], tissue scaffolds[12,22], protective clothing[23], wound dressing[24], and composite reinforcement[4].

Interestingly, one-pot approach branching of two different kind of monomers onto hydrolyzing collagen has not gained much attention. The novelty of this approach is to achieve a new thermally stable branched copolymer of collagen to be finally used in end uses such as fibers and fibrous assemblies which the sufficient chain entanglements improve the mechanical properties. To date, most of surface modifications of fibrous assemblies of collagen have

been performed as the post treatment that can affect the mechanical properties of the final product by increasing the glass transition temperature[25-28].

In this study, Collagen was solubilized by acid treatment. Subsequently, binary vinyl monomers of Methyl Methacrylate (MMA) and Ethyl Acrylate (EA) in the varied feed ratios were grafted onto acid soluble collagen (ASC) with the aim of benefiting from the firmness and the plasticizing capacity of the resulted copolymer. Methyl Methacrylate (MMA) and Ethyl Acrylate (EA) possess the same density and molar mass with a wide range of difference in the glass transition temperature of their components in polymer chain (Tg≈-5···105 °C). The grafting polymerization of comonomers feed ratios onto ASC in different fractions was investigated. This cost-effective method can be considered as a promising alternative for collagen based fibrous assemblies intending to exploit the advantages of the flexibility of amphiphilic branched copolymers.

The influence of important physicochemical properties of resulted copolymers on the shape of the DSC patterns, the conductivity value where the side chains of copolymer with low dielectric constant covalently bonded on ASC, initial viscosity and surface tension affected by the obvious wide molecular weight distribution of the achieved copolymer based on their composition dependencies copolymer was studied.

2- EXPERIMENTAL

2-1 Material

All chemicals and materials were purchased from Alfa Assar (Lancashire, UK) without any further treatment unless stated otherwise.

2-2 Preparation of Acid Soluble Collagen (ASC)

Acid soluble collagen (ASC) was prepared using collagen from calf skin (Devro Company Inc., UK) in 0.1 M acetic acid (AA) and distilled water to reach pH of 3±0.5. The mixture was incubated for 5 h at 45 °C in a 250-ml triple necked round bottom flask and a stirrer bar was added. This step was ended up in the suddenly increased temperature of 80 °C threshold when an aqua homogenous solution of ASC was achieved.

2-3 Graft polymerization onto ASC

Conventional free radical polymerization was used to synthesize the graft copolymers of distilled Methyl Methacrylate (MMA) and Ethyl Acrylate (EA) onto the solution of collagen in distilled water. In this step, the previous 250 ml triple necked round bottomed flask was applied as reaction vessel. N2 gas was served through the solution while stirring. Once the desired temperature was achieved, the gently addition of Benzoyl Peroxide (BPO) which was dissolved in Acetone (2 ml) as the initiator, was added to the reaction vessel within 10 min. Distilled MMA and EA monomers in the rates mentioned in Table 1, were then introduced via a syringe in 30 min. The temperature and reaction time after adding the initiator and the monomers were fixed in 80 °C and 60 min, respectively. The stirrer speed of 2400 rpm was constantly used during the polymerisation reaction. Precipitation of the graft copolymer occurred after 15 min of reaction time, developing a milky white solution in the aqua medium. To complete precipitation, the reaction mixture was then added to rapidly stirring excess cool methanol. The solution was filtered with a glass sinter filter and dried in vacuum oven at 25 °C until a constant weight was obtained.

As with any conventional free radical copolymerization reaction, P(MMA-co-EA) can be reached along with that of the desired copolymer (ASC-g-P(MMA-co-EA) owing to reactivity ratio effects or the separation of macromonomer from main and side chains [29-31]. To remove side products as they may adversely affect further processing, the simple isolation method of selective solvent extraction based upon the difference in the solubility was employed. Therefore, the grafted copolymer was extracted by repeated washings with hot water followed by acetone at room temperature to remove the associated ungrafted ASC and P(MMA-co-EA). The resulted copolymer was dried in a vacuum oven at room temperature until constant weight was achieved. The grafting parameters, such as the grafting percentage (GP) was calculated by the following equations:

$$GP = \frac{W2 - W0}{W0} * 100$$

where W_0, and W_2 were the weights of initial ASC and ASC-g-P(MMA-co-EA), respectively[33].

2-4 Characterization

To identify the polymeric content of grafted copolymer, FTIR Spectroscopy was carried out as an analytical technique used to scan test samples and observe chemical properties (Thermo Nicolet Avatar 370 DTGS). To prepare the disk, the sample in KBr is prepared in the range of 1%. The thermal analysis of achieved copolymers was performed by Differential Scanning Calorimeter (DSC, Mettler DSC 12E). Temperature range up to 300 °C with a heating rate of

10°C/min in nitrogen atmosphere was reached. Sensitivity curve was carried out performing thermal analysis on 8 mg of samples to evaluate changes in their thermal behavior. Inherent viscosities were determined using Brookfield DV-II+Pro Viscometer at a concentration of 0.1 $g.dL^{-1}$ in 1:1 acetone and acetic acid solution at (20 ± 0.2) °C. The conductivity value was determined using conductivity meter HANNA HI8733, applying copolymer solutions of different concentrations dissolved in Formic acid (FA). Surface tension studies of dilute to entangled solutions in FA were carried out by using KRŰSS Tensiometer.

3- RESULTS AND DISCUSSION

3-1 Preparation of Acid Soluble Collagen (ASC)

The solubility profile of ASC was presented in Fig. 1 by controlled parameters of temperature, rotation speed, time and pH. According to Muyonga et al, their result indicated that the significant wide molecular weight distribution achieved through the acid isolation process of insoluble collagen is partially inherent and is partly caused by the denaturation of α-chain in isolation process[34]. The pH value has an obvious influence on the solubility of the ASC, especially on the lager Average molecular weight segments, due to the repulsive force between chains when the net charge residues (negative or positive) increased if the pH was within the region lower or higher than the isoelectric point[35]. It has been also reported that the solubility of collagen in weak acid could reduce the denaturation of α-chains[34,36]. Therefore, it is expected that the structure of segments with higher average molecular weight could be more similar to that of its parent collagen. Although, the desired collagen with excellent solubility could be prepared according to their molecular weights[35], in this research due to preserving the considerable fraction of α-chain that has the potential to be denaturized by acid treatment, Acetic Acid was applied and pH value was kept in the region close to the isoelectric point.

3-2 Synthesis of the ASC-g-P(MMA-CO-EA) copolymers

This paper mainly presents the results of studies based on the copolymers achieved from the graft copolymerization of MMA-co-EA onto ASC by using BPO as initiator that is assisted from high agitation speed and controlled water content as the medium. Based on the facts that controlled agitation speed and water content can potentially inhibit

chaos in performing derivatization and/or grafting reactions and they obviously have a considerable influence on performance of the process while they haven't obtained much attention in the investigations carried out on graft polymerization of biopolymers in aqua medium. In this study, the agitation speed was suggested to be kept on the constant rotation of 2400 rpm to produce a uniform propagation of formed active radicals in the increasing viscosity of medium during the polymerization. Moreover, water is known as a proper solvent for ASC that opens the possibility of performing grafting reactions under homogeneous conditions, thus promising important advantages, such as a better control of the degree of conversion[1,37], a more uniform distribution of substituents throughout the polymerization and a higher conversion yield[29]. Thus, these two parameters are added to the previous parameters that were investigated in other papers. Fig. 1 shows the controlled condition of the solubilization and the polymerization reaction. The grafting polymerization was considered at the starting point of 300 minutes of Fig. 1. Table1 gives a summary of the amount of raw material used in the current study. In all experiments 11g ASC was used and the effect of varied MMA-co-EA feed ratios were examined.

The schematic of reaction mechanism for the graft copolymerization of ASC-g-P(MMA-co-EA) is demonstrated in Fig. 2. Chemical grafting involves the formation of active centers upon the ASC backbone. Once these centers are initiated and formed through thermal dissociation of BPO, copolymer chains of P(MMA-co-EA) start to grow on them, resulting in branches. It is reported that due to using BPO, possible reactions as following can be occurred: a)the chain-transfer reaction between the growing chains and the backbone, which forms active sites upon the backbone; b) the monomers add to active center upon the backbone to give a graft copolymer; c)the graft copolymer chain growing on the backbone in which termination of the process is likely to occur in one of the chain-transfer or combination reactions involving the growing chain radical on the backbone. Apart from the desired product, P(MMA-co-EA), ungrafted ASC and unconsumed monomers may be reached.

Fig. 3 shows the FTIR spectra of ASC-g-P(MMA-co-EA) as a prove of presents of amide groups in the samples where compared with ASC and P(MMA-co-EA). The main characteristic features were observed in the spectra. Collagen has several characteristic absorption bands known as amide A (3425 cm-1), amide B (2857- 2953 cm^{-1}), amide I (1621-1711 cm^{-1}), amide II 1466 cm-1 in the infrared region of the spectrum that are absorbed by ASC-g-P(MMA-co-EA). Characteristic bands of P(MMA-co-EA) were carbonyl (C=O) stretching vibration at 1720 cm^{-1}, CH stretching vibration at (2950- 3050 cm^{-1}) (asymmetric) and 2865 cm-1 (symmetric), C-O-C stretching at 1060 cm^{-1}, and C-O-C stretching

at 1260 cm^{-1} (asymmetric). The amide I adsorption originated largely from the C=O stretching vibration, is specifically sensitive to the secondary structure of the polypeptides[27]. For the amide B, the amide II region is affected by P(MMA-co-EA) absorptions, the amide A band and amide I were used as the reference peaks to confirm the presence of Collagen in the grafted copolymer in this analysis.

The growing branching on ASC incorporation correlates well with the feed ratios of MMA-co-EA in the reaction. The grafting performance proves the direct interaction of the feed ratio of co-monomers added onto ASC and the growing copolymer side chains. According to Table 1, a decrease in the grafting performance is evident at the highest feed ratio of S5 where the dominated co-monomers are more likely to be initiated and to be (co)polymerized solely in reaction. The deviations occurred in the grafting performance can be due to the steric effect and polarity effect of the combination of ASC and comonomers in acquis medium.

3-3 The influence of copolymer architecture on thermal behavior of grafted copolymers

The effect of branching from two different monomers of an elastic material (PEA) and of a brittle one (PMMA) with a wide broad range of glass transition temperature (Tg) onto the main polymer (ASC) was investigated to obtain a new flexible copolymer. The thermally stabilized final product within certain segments associated with the glass transition temperature (Tg) and the melting temperature (Tm) specifically, is essential to be considered.

Fig. 4, the DSC curve of the pure collagen showed an endothermic peak at 85 °C and 160 °C related to its melting point (Tm) and denaturation process, respectively. The melting temperature (Tm) increased in S1 possessing up to 50 wt (%) of P(MMA-co-EA) to 97 °C while Tg and Td values of this curve are reduced to 53 °C and 147 °C. This behavior can be affected by several parameters e.g. undergoing the slight denaturing of the backbone in the polymerization process when applying initiator in which may lower the average molecular weight. The enthalpy of denaturation (ΔHd), on the other hand, did not change significantly with the presence of higher fraction of synthetic polymers in S2-S5 samples and the Tg slightly increased to 61-65 °C. These results indicate that Collagen is thermally altered by the presence of P(MMA-co-EA) in the feed ratios mentioned in Table 1, as verified by the shifting of the melting peak towards higher temperatures, with no considerable changes on the enthalpy of denaturation and the glass transition temperature of the studied samples.

3-4 The influence of copolymer architecture on viscosity of grafted copolymers

The diluted solutions of 1 w/v% ASC-g-P(MMA-CO-EA) in FA were prepared to prevent significant reduction in hydrodynamic volume of solution. The changed level of branching displayed significantly different viscosities (η). Fig. 5 shows that over the shear rate range investigated, the grafted copolymer displayed Newtonian behavior. With the knowledge of having a higher absolute M_w, the highly-branched polymer displayed a higher viscosity compared to lower degrees of branching obtained in co-monomer feed ratio of S1.

3-5 The influence of copolymer architecture on surface tension of grafted copolymers

The surface (interfacial) tension of polymer solution plays a significant role in many applications using grafted polymers, such as coating, foaming and fiber spinning, [38]. This was attributed to entanglement couplings stabilizing and preventing the Raleigh instability. With the knowledge that the surface tension of diluted polymeric solutions tends to increase with increasing the Number Average Molecular Weight (M_n), the interfacial phenomena of diluted solutions were evaluated through applying the Nouy ring method. Fig. 6 demonstrates the surface tension of grafted copolymers from dilute to entangled concentration (C). Although in high concentrations, S2 represent the highest surface tension, all samples including ASC demonstrate a similar value in semi dilutes ($2\ \% \leq C \leq 6\ \%$). By contrast, in dilutes ($C < 2$), S4, S1, S2 show the higher values than S3, respectively. Regardless the C values, all samples tend to represent higher Surface tension that ASC, except diluted S5 that posed the lowest value. It is possibly due to wide molecular weight distribution occurred in random growth graft polymerization of S5. It can be seen that branching on ASC can slightly increase the surface tension of resulted copolymer solution.

3-6 The influence of copolymer architecture on the conductivity of grafted copolymers

The conductivity of the abovementioned solutions of ASC-g-P(MMA-co-EA) ($0.01 \leq C \leq 10$) was measured with the aim of comparing the conductivity of different branching levels in charge dynamics of the chains. The cationic characteristic of ASC is the responsible for the higher electric conductivity compared to the P(MMA-co-EA) with dielectric properties when they are dissolved in FA[39]. When P(MMA-co-EA) with low dielectric constant is grafted on the backbone owning amine and carboxyl group, is expected to react as a barrier against electron mobile phase

of the solution. In other words, for the solutions containing ASC-g-P(MMA-co-EA) that it was observed a sharp decrease of the conductivity value possibly due to hydrogen bond reduction resulted as an absolute consequence of graft polymerization on ASC and replacement of comonomers with low dielectric constant. As it can be seen in Fig. 7, the conductivity values of all studied samples are significantly lower than of the associated value of ASC. Interestingly, a meaningful arrangement on comonomer feed rates was observed, whereas S5 demonstrates the minimum conductivity value. This phenomenon can be evidenced by the density of branches on the backbone of ASC. The observations prove that the electrical behavior of ASC as a polyelectrolyte can significantly be affected by dielectric behavior of branched side.

4- CONCLUSIONS

In this study, it was shown that P(MMA-co-EA) can be grafted on hydrolyzing collagen in aqua medium. The various co-monomer feed ratios were investigated. The level of branching and the influence of branching was further investigated in relation to viscosity, surface tension and conductivity values of the grafted copolymers at different branching levels. The thermal behavior of all samples was affected by grafting polymerization. The shear rate dependence of viscosity for highly branched segmented copolymer was studied and their related Newtonian behavior was investigated. Whilst the surface tension of the studied solutions underwent a slight increase by branching, viscosity of the diluted solutions was significantly affected by increasing the comonomer feed ratio in which high viscosity was observed in S4 with the highest grafting percentage. The conductivity value of the solutions received a considerable decrease due to low dielectric behavior of P(MMA -co-EA) participated in the reactions. The increasing monomer feed ratio showed negligible change in conductivity value of the resulted copolymers.

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